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Effect of watering frequency and feed on blood biochemical parameters in crossbred calves

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Abstract
The calves were randomly assigned to four watering frequency (T_1, T_2, T_3 & T_4) and two feeding treatments (F_1&F_2), a total of eight treatments, on the basis of sex and body weight viz., T_1F_1: watering frequency *ad lib*. with 50:50 mixture of legume (soyabean) straw and cereal (wheat) straw, T_1F_2: watering frequency *ad lib*. with Jowar hay, T_2F_1: watering frequency once a day with 50:50 mixture of legume (soyabean) and cereal (wheat) straw, T_2F_2: watering frequency once a day with Jowar hay, T_3F_1: watering frequency twice a day with 50:50 mixture of legume and cereal straw, T_3F_2: watering frequency twice a day with Jowar hay, T_4F_1: watering frequency thrice a day with 50:50 mixture of legume and cereal straw and T_4F_2: watering frequency thrice a day with Jowar hay. In all treatments, fixed quantity of concentrate and green fodder were also offered to calves. Twenty four crossbred calves were used for 98 days experimental period. Blood samples were collected three times i.e., 0, 45 and 90 days of experiment from the jugular vein of each experimental animal under aseptic precaution. Haemoglobin and PCV (%) was significantly (p<0.05) higher in T_2 and T_3 as compared to T_1. However, haemoglobin and PCV of experimental animals in T_4 group was at par with remaining treatments. Average BUN, Uric acid, Creatinine, SGPT and SGOT was found to be higher in once day watering frequency (T_2) as compared to other groups. A statistically non-significant difference in mean of haemoglobin, PCV, creatinine, glucose, total protein, SGPT and SGOT in two feeding treatments. Jowar hay fed calves had significant higher (p<0.05) in BUN and uric acid level than mixture of legume and cereal straw. All blood parameters were with in normal range except BUN, uric acid and creatinine in once watering frequency.

Keywords: Blood biochemical parameters, crossbred calves, watering frequency, feed, haemoglobin

Introduction
Livestock provides supplementary income to families which are dependent on agriculture for their lively hood. In dairy farming, care and management of calf is crucial as they are the future dairy animals. Careful rearing and scientific management of livestock during their early growth period is the main basis for good animal husbandry. Water is one of the vital nutrients in all feed stuffs beside fat, carbohydrate, protein, mineral and vitamin. Among all nutrients, water is the most ignored nutrient in the field of livestock production. It sustains the health and integrity of each cell and forms the basis of fluids in the body. It has been reported that loss of water exceeding more than 10% is fatal and can results in death whereas animal survives even when it loses all its body fat and one-third of body protein (ICAR, 2013) [6]. Water requirement for calves is affected by many factors, including age, type of feedstuffs (e.g., concentrate, hay, silage or fresh forage), water consumption, including water quality, drinker design, dry matter intake, weather conditions, and housing (Quigley *et al.*, 1998; Hepola *et al.*, 2008) [15, 5]. Watering frequency or water restriction also affects hematological and biochemical profile of blood. Water restriction significantly increases hemoglobin, BUN concentration, creatinine, uric acid and total protein level in blood (Gajarlawar, 2014; Benatallah *et al*., 2019; Sahana *et al*., 2020) [3, 1, 16]. Therefore, this experiment was planned to evaluate the effect of watering frequency and feed on blood biochemical parameters of crossbred calves with above facts in mind.

Material and Methods
The experiment was conducted at Livestock Research Station (LRS), AAU, Anand for 98 days as per guideline of Institutional Animal Ethics Committee (Sanction No. 300/LPM/2019) of College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand, Gujarat. Total 24 crossbred male and female (HF x Kankrej) calves were allotted to eight groups (each treatment had 1 male and 2 female calves) with average similar body weight.

Calves were subjected to four watering frequency (T_1, T_2, T_3 & T_4) and two feeding treatments (F_1 & F_2), a total of eight treatments viz., T_1F_1: watering frequency *ad lib*. with 50:50 mixture of legume (soyabean) straw and cereal (wheat) straw, T_1F_2: watering frequency *ad lib*. with Jowar hay, T_2F_1: watering frequency once a day with 50:50 mixture of legume (soyabean) and cereal (wheat) straw, T_2F_2: watering frequency once a day with Jowar hay, T_3F_1: watering frequency twice a day with 50:50 mixture of legume (soyabean) and cereal (wheat) straw, T_3F_2: watering frequency twice a day with Jowar hay, T_4F_1: watering frequency thrice a day with 50:50 mixture of legume (soyabean) and cereal (wheat) straw and T_4F_2: watering frequency thrice a day with Jowar hay.

All experimental animals were kept under iso-managerial conditions in well ventilated hygienic shed and were provided clean drinking water and feed as per treatment. Watering of experiment animals were divided into four treatment group viz., T_1 calves was offered *ad lib*. water, T_2 calves was offered once a day water at 10 a.m., T_3 calves was offered twice a day water at 10 a.m. and 9 p.m. and T_4 calves was offered thrice a day at 10 a.m., 3 p.m. and 9 p.m. *Ad lib*. mixture of legume (soyabean) and cereal (wheat) straw (50:50) or chaffed Jowar hay were offered at 3:00 pm to fulfill their nutrient requirement as per treatment schedule. To meet nutrient requirements (ICAR, 1998) [6], all calves were individually fed green hybrid napier and concentrate.

Blood Profile

The blood samples were collected three times i.e., at 0, 45 and 90 days aseptically in the morning from the jugular vein in EDTA vacuttee. Hematological parameters viz., Hemoglobin (HB) and Packed Cell Volume (PCV) were estimated immediately using blood auto analyzer. While for biochemical estimation, 4-5 ml blood sampling was collected in vacutainer without anticoagulant. Blood samples after collection were kept undisturbed for 3-4 hours and serum was separated out by centrifugation at 3000 rpm for 30 minutes. Serum samples were stored in deep freeze at -20 ºC until analysis of various biochemical profiles. Biochemical profiles viz., Serum glucose, Serum BUN, Serum uric acid, Serum creatinine, Serum total protein, Serum Glutamic Pyruvic Transaminase and Serum Oxaloacetic Transaminase were analyzed using standard Coral diagnostic kits manually. Observations of various parameters recorded and derived during the experimental period were tabulated and statistically analyzed using Randomized Block Design (Factorial) as stated by Snedecor and Cochran (2002) [17].

Results and Discussion

Haemoglobin

Average values of blood haemoglobin (g/dL) of the crossbred calves is presented in table 1. Overall average haemoglobin of experimental calves was found to be 8.92±0.10 g/dL. The haemoglobin was found to be in normal range of 8-15 g/dL (Kahn, 2005) [11]. Irrespective of source of feed, Haemoglobin was significantly (p<0.05) higher in T_2 and T_3 as compared to T_1. However, haemoglobin of experimental animals in T_4 group was at par with remaining treatments. The increased Hb concentration in twice a day watering frequency group might be due to development of haemoconcentration by consuming less water. Ignoring watering frequency, haemoglobin was statistically non-significant in mixture of legume & cereal straw (F_1) and Jowar hay (F_2). Fluctuation in the HB content in the blood of calves due to watering frequency and not by feed source. The results in present study are in agreement with Gajarlawar (2014) [3] where they had found significant result on blood haemoglobin in different watering frequency in Deoni cows. Average Hb content in blood was 11.03, 11.16 and 11.02 g/dL in *ad lib*. (T_1), twice a day (T_2) and thrice a day (T_3) watering frequency, respectively. Haemoglobin was significantly (p<0.05) higher in T_2 as compared to T_1 and T_3, whereas T_1 and T_3 was statistically similar. In contrast to present study, Gottardo *et al.* (2002) [4] reported that blood haemoglobin (g/dL) in Polish Friesian calves was 9.48 and 9.53 in no water (only milk replacer diet) and *ad lib*. watering, respectively which was statistically non-significant.

Table 1: Mean±S.E. of haemoglobin (g/dL) in crossbred calves

	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	8.58±0.12	8.50±0.25	8.93±0.33	9.47±0.40	9.67±0.21	8.58±0.30	8.96±0.21	8.68±0.24	9.03±0.13	8.81±0.16
Overall mean	8.54^{A}±0.13		9.20^{B}±0.26		9.12^{B}±0.22		8.82^{AB}±0.16		8.92±0.10	

Means with dissimilar superscripts in a row (A, B) differ significantly for watering frequency (p<0.05)

PCV (%)

Significantly (p<0.05) higher was found in PCV levels in T_2 and T_3 as compared to T_1 (table 2). However, PCV in T_4 group was at par with remaining watering frequencies. Results of Sahana *et al*. (2020) [16] are similar with present study where eighteen Indigenous sheep were divided into three treatment groups i.e. T_1 (thrice a day), T_2 (twice a day) and T_3 (Once a day) watering. Average PCV (%) content in blood was 31.56±0.33, 31.97±0.45 and 32.99±0.22 in T_1, T_2 and T_3 group, respectively. The PVC (%) was significantly (p<0.05) higher in once a day and twice a day watering.

Table 2: Mean±S.E. of PCV (%) in crossbred calves

	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	27.02±0.39	26.78±0.79	28.14±1.04	29.82±1.26	30.45±0.67	27.02±0.94	28.21±0.69	27.34±0.76	28.46±0.41	27.74±0.52
Overall mean	26.90^{A}±0.43		28.98^{B}±0.82		28.74^{B}±0.70		27.77^{AB}±0.51		28.10±0.32	

Means with dissimilar superscripts in a row (A, B) differ significantly for watering frequency (p<0.05)

BUN

Irrespective of source of feed, serum BUN content was significantly (p<0.05) higher in T_2 and T_3 as compared to T_1 and T_4. This indicated that, there was increase in BUN content in the blood of crossbred calves when watering frequency was restricted to once a day and twice a day whereas, serum BUN

content in the blood of calves from *ad lib*. and thrice a day watering frequency remain unaffected. Regardless of watering frequency, serum urea (mg/dL) was significantly (p<0.05) higher in calves fed Jowar hay (F_2: 29.58±0.56) as compared to mixture of legume & cereal straw (F_1: 27.18±0.74). Similary, plasma concentrations of urea was significantly (p<0.05) increased in 50% water restricted dairy cows as compared to cow offered *ad lib*. water as reported by Burgos *et al*. (2001) [2]. The results in present study are also in agreement with Gajarlawar (2014) [3] where they found (p<0.05) significant effect on blood urea nitrogen in different watering frequency in Deoni cows. Benatallah *et al*. (2019) [1] also observed that BUN content significantly (p<0.05) increased when lactating cows were subjected to 25% and 50% water restriction as compared to *ad lib* watering.

Table 3: Mean±S.E. of BUN (mg/dL) in crossbred calves

	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	23.47±0.84	25.78±0.72	31.56±0.59	32.80±0.49	30.67±0.69	34.40±0.89	23.02±0.56	25.33±0.58	27.18^{a}±0.74	29.58^{b}±0.76
Overall mean	24.62^{A}±0.60		32.18^{B}±0.40		32.53^{B}±0.69		24.18^{A}±0.48		28.38±0.54	

Means with dissimilar superscripts in a row (A, B) differ significantly for watering frequency and (a, b) for feed source differ significantly (p<0.05)

Uric acid

The overall average serum uric acid (mg/dL) of experimental calves was found to be 5.06±0.17 (table 4). Ignoring feed source, uric acid was significantly (p<0.05) higher in once a day watering frequency (T_2) followed by T_3, T_4 and T_1, however T_4 was at par with T_1. Regardless of watering frequency, uric acid (mg/dL) was significantly (p<0.05) higher in calves fed Jowar hay (F_2) as compared to mixture of legume & cereal straw (F_1). Plasma uric acid concentration (mg/dL) of Indigenous sheep significantly (p<0.05) increased in once a day watering frequency as compared to sheep maintained in twice a day watering frequency and *ad lib*. watering as per the study of Sahana *et al*. (2020) [16]. The pattern of watering frequency with uric acid concentration change was in accordance with present findings.

Table 4: Mean±S.E. of uric acid (mg/dL) in crossbred calves

	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	3.21±0.12	4.15±0.25	6.83±0.15	6.59±0.20	5.77±0.34	6.17±0.11	3.77±0.18	3.96±0.38	4.90^{a}±0.26	5.22^{b}±0.23
Overall mean	3.68^{A}±0.17		6.71^{C}±0.12		5.97^{B}±0.18		3.87^{A}±0.20		5.06±0.17	

Means with dissimilar superscripts in a row (A, B) differ significantly for watering frequency and (a, b) for feed source differ significantly (p<0.05)

Creatinine

Statistical analysis of data revealed significantly (p<0.05) higher serum creatinine in once a day (T_2) in comparison to *ad lib*. (T_1), twice a day (T_3) and thrice a day (T_4) watering frequency groups. The result of current study is in agreement with Kaneko *et al*. (2008) [9], who reported serum creatinine of bovine to be 0.6-1.8 mg/dL under normal level. The serum creatinine concentration (mg/dL) of indigenous sheep significantly (p<0.05) increased in once a day watering as compared to sheep maintained in twice a day watering and *ad lib*. as per the study of Sahana *et al*. (2020) [16] which again supports the results of present findings. Benatallah *et al*. (2019) [1] also observed that creatinine concentration in blood was significantly (p<0.05) increased when lactating cows were subjected to 25% and 50% water restriction as compared to *ad lib* watering.

Table 5: Mean±S.E. of creatinine (mg/dL) in crossbred calves

	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	0.85±0.07	1.08±0.12	1.44±0.12	1.51±0.12	1.13±0.11	1.08±0.14	0.96±0.03	0.98±0.05	1.10±0.05	1.16±0.06
Overall mean	0.96^{A}±0.07		1.48^{B}±0.08		1.10^{A}±0.08		0.97^{A}±0.03		1.13±0.04	

Means with dissimilar superscripts in a row (A, B) differ significantly (p<0.05) for watering frequency

Glucose

Overall average serum glucose was 69.99±0.91 mg/dL in table 6. Glucose level in the blood during this experiment was found to be in normal range of 40-100 mg/dL (Khan, 2005) [11]. Serum glucose (mg/dL) of experimental animals in T_1, T_2 and T_4 was significantly (p<0.05) higher as compared to T_3 group. On the basis of different feed source (F_1&F_2), serum glucose was statistically similar. Findings of present study were in line with Burgos *et al*. (2001) [2] who reported that plasma glucose increased in *ad lib*. watering as compared to 50% water restricted in dairy cows. Contrary to the present findings, Gajarlawar (2014) [3] documented that no significant difference in blood glucose of Deoni cows in *ad lib*., twice a day and thrice a day watering frequency.

Table 6: Mean±S.E. of glucose (mg/dL) in crossbred calves

Period	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	74.00±3.18	74.91±2.02	71.38±2.88	68.81±3.02	64.73±2.44	63.36±0.96	68.07±1.22	74.68±1.46	69.54±1.35	70.44±1.28
Overall mean	74.45^{B}±1.83		70.09^{B}±2.05		64.04^{A}±1.28		71.38^{B}±1.22		69.99±0.91	

Means with dissimilar superscripts in a row (A, B) differ significantly (p<0.05) for watering frequency

Total protein

An experiment revealed that watering frequency and different feed source had no effect on blood total protein in crossbred calves. The total protein level in this experiment was found to be in normal range 6.0-8.0 g/dl (Khan, 2005) [11]. The finding of present study goes with Kheir and Ahmed (2008) who had concluded that serum protein was no significant effect in Sudanese Desert goats on water restriction. Gajarlawar (2014) [3] also indicated non-significant serum total protein in different watering frequency in Deoni cows. Kapadiya *et al.* (2019) [10] reported that overall average total protein was 6.13±0.05 g/dL of Jowar hay roughage feeding. This report was lower than present study.

Table 7: Mean±S.E. of total protein (g/dL) in crossbred calves

Period	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	6.28±0.09	6.55±0.19	6.43±0.23	6.62±0.17	6.58±0.10	6.54±0.14	6.42±0.10	6.49±0.14	6.43±0.07	6.55±0.08
Overall mean	6.42±0.11		6.52±0.14		6.56±0.08		6.45±0.08		6.49±0.05	

Means with dissimilar superscripts in a row (A, B) differ significantly (p<0.05) for watering frequency

SGPT

The overall average SGPT level of experimental calves was found to be 35.16±0.26 U/L (table 8). The values of SGPT (32.13-39.32 U/L) were within normal range (Mamun *et al*., 2013). Irrespective of feed source, SGPT was significantly (p<0.05) higher in T_2 followed by T_1 and T_4, however, the value of T_3 was at par with T_1 and T_2 groups. All the values of SGPT were under normal level as reported by Kaneko *et al*. (2008) [9]. As per the study of Kshatriya (2009) [13], overall mean SGPT of crossbred calves was 41.19±0.67 U/L in twice a day drinking water with feeding of non-conventional concentrate mixture.

Table 8: Mean±S.E. of SGPT (U/L) in crossbred calves

Period	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	34.35±0.58	35.55±0.36	37.27±0.80	35.42±0.76	35.84±0.73	35.43±0.42	33.57±0.69	33.81±0.95	35.26±0.41	35.05±0.33
Overall mean	34.95^{B}±0.36		36.34^{C}±0.58		35.64^{BC}±0.41		33.70^{A}±0.57		35.16±0.26	

Means with dissimilar superscripts in a row (A, B) differ significantly (p<0.05) for watering frequency

SGOT

Regardless of feed source, mean SGOT was significantly (p<0.05) higher in T_2 and T_4 as compared to T_1. However, SGOT of experimental animals in T_3 group was at par with remaining treatments. SGOT level in this experiment was found to be in normal range of 47.60-61.02 U/L (Kamala *et al*., 2016) [8], indicating watering frequency and feed source had no major damaging effect on liver and or muscle of crossbred calves. As per the study of Kshatriya (2009) [13], overall mean SGOT of crossbred calves was 81.41±1.98 U/L in twice a day drinking water with feeding of non-conventional concentrate mixture. This value was higher than present findings. Similarly, Kapadiya *et al.* (2019) [10] reported that overall average plasma SGOT was 59.42±0.76 U/L of Jowar hay roughage feeding.

Table 9: Mean±S.E. of SGOT (U/L) in crossbred calves

Period	T1		T2		T3		T4		Mean	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Mean	60.02±0.94	55.80±1.53	59.66±1.09	61.23±0.69	58.55±1.07	60.85±1.41	59.97±1.34	60.25±0.83	59.55±0.54	59.53±0.65
Overall mean	57.91^{A}±1.01		60.45^{B}±0.65		59.70^{AB}±0.90		60.11^{B}±0.76		59.54±0.43	

Means with dissimilar superscripts in a row (A, B) differ significantly (p<0.05) for watering frequency

Conclusion

Experimental animals of all treatment groups well healthy with considering to hematological and biochemical profile. Even once day watering frequency showed similar hematological parameters, but BUN, uric acid and creatinine had level high in once day watering frequency.

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